(dopaminergic, cholinergic, and motor neurons), cardiomyocytes, and hepatocytes, and will facilitate development of in vivo cell fate conversion as a therapeutic strategy for neurological diseases and other disorders.

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Aspp1: A Guardian of Hematopoietic Stem Cell Integrity

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Eliminating hematopoietic stem cells (HSCs) with DNA damage is necessary to maintain the homeostasis of HSCs, but the mechanisms underlying this apoptotic elimination are unclear. Now in *Cell Stem Cell*, Yamashita et al. (2015) show that Aspp1 coordinates with p53 to protect HSC pool integrity, guarding against hematological malignancies.

Tissue-specific stem cells, including hematopoietic stem cells (HSCs), are long-lived, often quiescent cells with self-renewal properties that maintain organ homeostasis. Although quiescence is thought to protect cells from some endogenous stresses, it also presents challenges for the cells in terms of maintaining genomic integrity. Damaged DNA accumulates in quiescent HSCs because they are obliged to use error-prone non-homologous recombination mechanism (also called non-homologous end joining, or NHEJ) to repair their damaged DNA. In contrast, cycling HSCs repair DNA damage via error-proof homologous recombination (HR), but DNA replication also generates random mutations. Hence, regardless of the proliferation status of HSCs, there must be mechanisms that can effectively eliminate damaged cells to prevent hematological malignancies. The tumor suppressor p53 is known to be involved in DNA-damage-induced apoptosis in both human and mouse HSCs, but little is known about how this property of p53 is gated in response to diverse forms of genotoxic stress to control HSC viability or elimination. In this issue of *Cell Stem Cell*, Yamashita et al. (2015) reveal Aspp1 as a molecular guardian that protects mouse HSC integrity in both p53-dependent and -independent manners.

The importance of p53 in suppressing hematopoietic malignancy is supported by a recent finding that *p53* mutation precedes the diagnosis of acute myeloid leukemia (AML), with therapy-related AML resulting from clonal expansion of pre-existing mutant-*p53*-containing HSC clones (Wong et al., 2015). Importantly, that study also showed that ~50% of elderly healthy individuals carry one copy of mutant *p53* gene, illustrating that additional genetic alterations are required for transformation. Therefore, there is great interest in identifying additional proteins that synergize with p53 to defend against hematological malignancies. ASPP1, a member of the evolutionarily conserved apoptosis stimulating proteins of p53 (ASPP) family, is one such protein. ASPP1, and the related ASPP2, binds p53 and its siblings, p63 and p73, and selectively activates their pro-apoptotic functions (Bergamaschi et al., 2004). Transgenic mouse studies have established Aspp2 as a haploinsufficient tumor suppressor and an activator of p53 (Vives et al., 2006). ASPP1 has been identified as an enhancer of apoptosis in human cord blood stem cells (Milyavsky et al., 2010), but little is known about the tumor suppressive function of ASPP1 in adult HSCs in vivo.

Yamashita et al. first confirmed previous findings that, after genotoxic stress, p53 is expressed at higher levels in populations enriched for hematopoietic stem and progenitor cells (HSPCs) compared to those enriched for myeloid progenitor cells, and they also observed that Aspp1 is more highly expressed in defined HSC populations than in other hematopoietic cell types (Yamashita et al., 2015). In *Aspp1*-deficient mice, the authors found an elevated number of white blood cells and bone marrow myeloid mononuclear

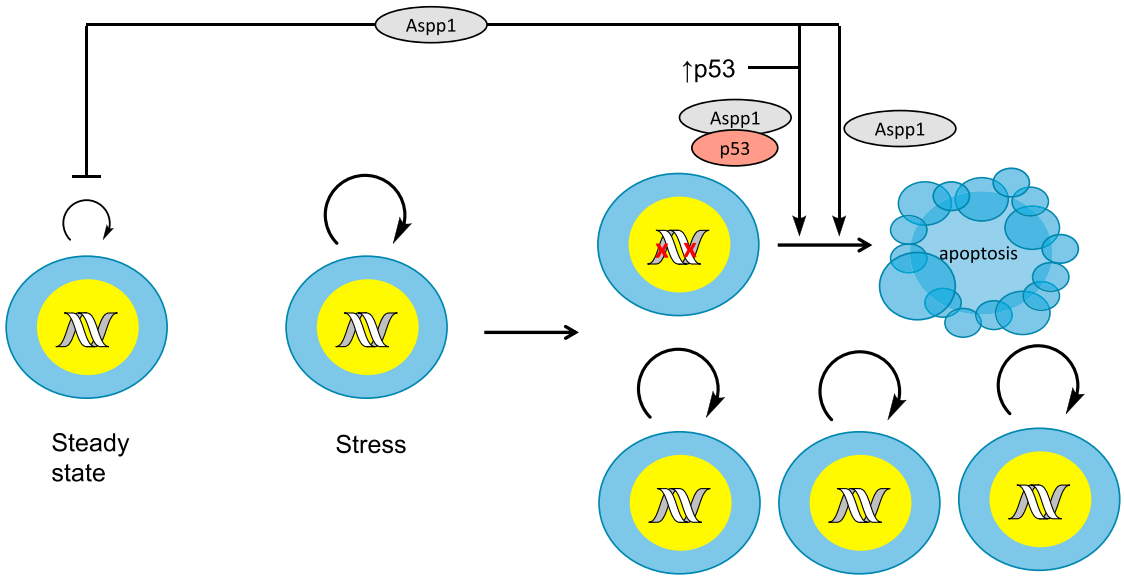

Figure 1. The Dual Role of Aspp1 in HSCs

Under steady-state conditions, Aspp1 promotes quiescence (left). Stress such as ionizing radiation prompts HSCs to re-enter the cell cycle (middle) and they thus become prone to acquire DNA damage during cycling. Aspp1 works both together with and independently of p53 to activate the apoptotic response in heavily DNA-damaged HSCs (right), while non-damaged HSCs remain proliferative (bottom right).

cells, but also 1.5- to 2-fold higher levels of HSC-containing populations (specifically, lineage$^-$Sca-1$^+$c-Kit$^+$ cells [LSKs] and long-term HSCs [LT-HSCs]). This suggests that Aspp1 negatively regulates HSC proliferation and/or survival. When *Aspp1*$^{-/-}$ HSCs were subjected to serial competitive transplantation, compared to wild-type cells they survived significantly longer and showed reduced apoptosis and upregulated genes associated with quiescence. A higher proportion of *Aspp1*$^{-/-}$ HSCs also remained quiescent and there was less cell death after genotoxic stress induced by 5-fluorouracil administration. Similar results were observed in cultured HSCs from irradiated mice: unlike wild-type HSCs, *Aspp1*$^{-/-}$ HSCs were able to survive even with a large number of DNA damage foci and expressed lower levels of pro-apoptotic genes. Importantly, no increase of apoptosis was observed in steady-state HSCs, indicating that Aspp1 only promotes apoptosis in response to stress.

To investigate the relationship between p53 and these Aspp1-dependent effects, the authors established mice with *Aspp1*$^{-/-}$*p53*$^{-/-}$ HSC populations. Although the number of HSCs in these mice was increased, levels of apoptosis were comparable to those observed in wild-type animals. In serial competitive transplantation experiments, *Aspp1*$^{-/-}$ *p53*$^{-/-}$ HSCs displayed enhanced engraftment compared with HSCs from *Aspp1*$^{-/-}$ and *p53*$^{-/-}$mice. The double-deficient mice showed a higher number of HSCs with severely damaged DNA than *p53*$^{-/-}$ or *Aspp1*$^{-/-}$ mice. Mice transplanted with *aspp1*$^{-/-}$ HSCs did not develop spontaneous lymphoma or leukemia, but the additional deletion of *Aspp1* significantly increased the lethality of leukemia lymphoma in mice receiving *p53*$^{-/-}$ HSC transplants. Together, these findings suggest that Aspp1 has roles in the regulation of HSC renewal and the protection against malignancy, which are both dependent on and independent of p53.

Aspp1 seems to have distinct roles in steady-state versus stressed HSCs: in steady-state conditions, Aspp1 promotes quiescence and inhibits differentiation into myeloid progenitors. It is not known how Aspp1 performs this function. Upon stress, Aspp1 promotes apoptosis in HSCs with high levels of DNA damage, preventing the accumulation of both quiescent and self-renewing DNA-damaged HSCs (Figure 1). Interestingly, elevated p53 induces senescence in primary fibroblasts but apoptosis in highly proliferative cancer cells. The precise mechanisms underlying these distinct p53-mediated effects remain largely unknown, but might be due to elevated ASPP1 and ASPP2 expression in proliferating cells. ASPP1 and ASPP2 are transcriptional targets of E2F1, a key promoter of cell cycle entry (Fogal et al., 2005). As stress induces HSC proliferation, it is possible that elevated expression of ASPP1 may set a threshold for inducing p53-mediated apoptosis in proliferating HSCs. It will be interesting to investigate how ASPP1 increases susceptibility to both p53-dependent and p53-independent apoptosis when the HSCs enter the cell cycle after genotoxic stress.

In terms of human hematological malignancies, it is important to note that—unlike in solid tumors—the *TP53* gene is mutated infrequently, except in certain tumor subtypes with complex karyotypes or after chemotherapy (Holmfeldt et al., 2013; Wong et al., 2015). How can this be reconciled with the observed role of p53 in eliminating HSCs with DNA damage? One possibility is that the relatively low levels of somatic mutations in leukemias and lymphomas are not sufficient to activate p53-dependent apoptosis, and malignant cells evade p53-independent apoptosis through upregulation of anti-apoptotic proteins such as Bcl-2 (Tsujimoto et al., 1985). It is also possible that the role of p53 in maintaining HSC

self-renewal might provide selective pressure to retain functional p53. Since ASPP1 is a guardian of HSC integrity, similar pressure may also exist to maintain ASPP1 in hematopoietic malignancies. Interestingly, genomic data suggest that *ASPP1* is not commonly mutated or deleted in human hematological cancers, but frequent downregulation of *ASPP1* mRNA, such as through promoter methylation, has been observed (Agirre et al., 2006). In the light of the findings of Yamashita et al., it would therefore be informative to assess the mutation and expression status of p53 and ASPP1 together in hematological malignancies.

More broadly, the discovery of p53-dependent and -independent roles of Aspp1 in HSCs highlights the need to investigate roles of the ASPP proteins beyond their regulation of p53. Therefore, the work of Yamashita et al. both unveils an important aspect of HSC quality control regulation and provides insights into the control of apoptosis.

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Back to 2D Culture for Ground State of Intestinal Stem Cells

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Generating highly uniform clonogenic stem cell populations has been remarkably difficult with stem cells from human small and large intestine. A recent report by Wang et al. (2015) demonstrated homogeneous expansion of human fetal intestinal stem cells, providing a new culture system to understand self-renewing mechanisms of intestinal stem cells.

Intestinal epithelium is a rapidly self-renewing tissue, and its proliferation is confined to the bottom of crypts where the intestinal stem cell niche exists. Rapidly dividing intestinal stem cells produce daughter cells that migrate out of the crypt niche and differentiate into transit amplifying cells. Recent research identified niche factors that emanated from the crypt bottom and regulate intestinal stemness. These niche factors include Wnt, EGF, Notch ligands, and BMP/TGF-b pathway inhibitors (Sato et al., 2009; Sato and Clevers, 2013). Under the presence of defined niche factors, intestinal stem cells are capable of forming organoids and indefinitely self-renew and produce both intestinal stem cells and all types of differentiated intestinal epithelial cells. In the organoid culture system, intestinal stem cells also require niche signals from adjacent differentiated epithelial cells, such as Wnt or Notch signaling from Paneth cells, which precludes the generation of a uniform clonogenic intestinal stem cell population (Figure 1). A combination of two small molecule inhibitors, which activate Wnt and Notch signaling, mitigates dependency of Lgr5+ stem cells on differentiated niche cells, resulting in increased clonogenicity of mouse intestinal stem cells, by up to 40% (100-fold greater than standard culture conditions) (Yin et al., 2014). However, the combination of chemical inhibitors has not been applied to human intestinal epithelium.

Wang et al. now developed a new culture system that enables fetal human intestinal stem cells to indefinitely propagate as uniform highly clonogenic stem cells, termed "ground state" stem cells of the human intestine (Wang et al., 2015). "Ground state" has been originally used to describe the most primitive state of embryonic stem cells (ESCs) and has been useful for understanding the regulation of pluripotency (Hackett and Surani, 2014). Here, in analogy with the ground state of ESCs, Wang and colleagues establish the elemental state of tissue stem cells in vitro. They exploited feeder layer culture methods originally